THE MITOCHONDRIA OF A PROTOZOAN (OPALINA) AND THEIR BEHAVIOUR DURING THE LIFE CYCLE

by

E. S. HORNING

(Department of Zoology, University of Melbourne.)

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I.

In this paper I wish to record a series of observations made upon the mitochondria of a common infusorian Opalina: the change which they undergo during the life cycle of the organism, and the part they appear to play in its metabolism.

Life Cycle of Opalina.

In the Australian Opalinas the stage which is best known and most usually found is the large binucleate condition of the parasite. Binary fission of the organism, either transverse or longitudinal, occurs at times, more especially towards the reproductive period of the adult frog; it is not accompanied by nuclear division, so that the resulting organisms are uninucleated. These uninucleate forms may develop into typical binucleate forms again, or on the other hand, may proceed, probably after undergoing rapid fission, to form minute cysts. The spherical cysts are passed to the exterior with the faeces, and are then eaten by young tadpoles. In the rectum of these young tadpoles each cyst gives rise, according to Metcalf (3), to a single organism, closely resembling the pre-encystment form, and this gives rise, by repeated fission to the gametes, the male being recognized by the presence of a long tail. After fertilization a uninucleate form develops from the zygote; this grows in size, its nucleus divides, and the typical binucleate form is again produced.

Method.

In order to examine the behaviour of the mitochondria during this life cycle, the rectal contents of starved frogs *(Hyla aurea)*, which usually swarm with Opalinids, were fixed in osmo-chromic fixative, concentrated by centrifuging, and

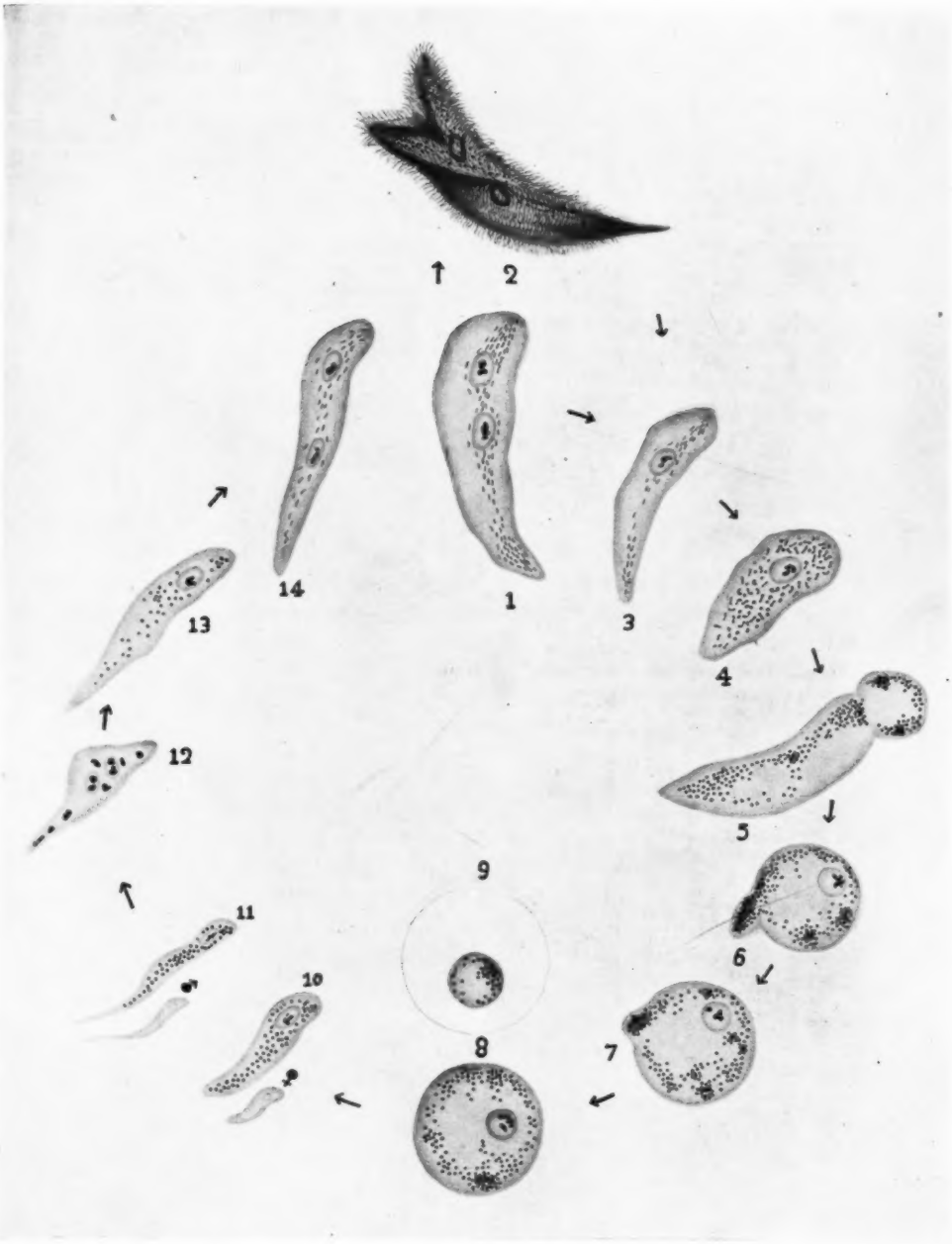
eventually embedded in mass in paraffin and sectioned. The sections were stained with iron-haematoxylin.

If such sections are examined with high magnifications the cytoplasm of the organism is seen to contain a very large number of rods, sometimes straight, but generally bent into an incomplete circle. Their nature was at first a matter of speculation; they certainly have the appearance of bacteria, but their subsequent behaviour during the life cycle shows quite clearly that they cannot be symbiotic organisms. If the Opalinas are killed with Bouin's fixative, instead of with osmo-chromic, the bodies can be stained only very faintly. In this they resemble the mitochondria of metazoa, which are similarly rendered almost non-stainable by fixatives which contain acetic acid. Furthermore, I find that if Opalinids are placed for a considerable time in Ringer's fluid (frog's), the Ringer fluid has a deleterious effect upon them; they fuse with one another, and in the place of numerous long, bent, filamentous bodies, we now find, on sectioning and staining, a relatively small number of large, spherical globules (figure 18). Very similar postmortem effects are to be observed with the mitochondria of metazoa, merely by letting the tissue stand for several hours after the killing of the animal. Granules formed in this way from the filamentous mitochondria of the pancreas of the guinea-pig are shown in figure 19. Further, I find that if osmo-chromic preparations are examined unstained the filaments appear a very faint brown colour, similar to the colour of lipoidal material after treatment with osmic acid. In this respect also the filaments resemble the mitochondrial bodies of metazoa. From these various considerations I believe then that the rod-like cytoplasmic constituents of Opalina are of the same nature as the mitochondria of higher animals and plants.

DESCRIPTION OF PLATE 1.

Sections of Binucleate Opalina at various stages of life cycle. Osmo-chromic fixation, stained with Heidenhain's iron-haematoxylin.

Fig. 1. Adult binucleate Opalina, showing filamentous mitochondria. (X 1600.)
Fig. 2. Binucleate Opalina (drawn from life) undergoing longitudinal fission. Note the mitochondria concentrating at the anterior and posterior ends. (X 1600.)
Fig. 3. Uninucleate form, showing filamentous mitochondria.
Fig. 4. Section of Opalina prior to encystment in Ringer's fluid. Note mitochondria undergoing fission. (X 1600.)
Fig. 5. Opalina undergoing encystment in Ringer's fluid. Note spherical mitochondria, which have arisen by fission of the rod-shaped mitochondria of previous stages. (X 1600.)
Fig. 6. Later stage (Ringer's solution) of encystment, showing the rounded bodies (mitochondria). (X 1600.)
Fig. 7. Encystment almost complete; cytoplasm containing spherical bodies (mitochondria). (X 1600.)
Fig. 8. Encystment phase complete (from Ringer solution). (X 1600.)
Fig. 9. Natural cyst from rectum of tadpole. Note the appearance of spherical bodies (mitochondria) as in Ringer's cyst.
Fig. 10. Female and male gametes. Note spherical bodies (mitochondria). The small figures represent the organism drawn to magnification of 1600.
Fig. 12. Conjugation of male and female gametes, showing apparent fusion of maternal and paternal mitochondria.
Fig. 13. Zygote, uninucleate phase, showing numerous rounded granules in cytoplasm.
Fig. 14. Adult binucleate phase, present in tadpole before metamorphosis; showing the filamentous mitochondria which have arisen from the spherical granules.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The Behaviour of Mitochondria.

In the adult binucleate organism the mitochondria are to be seen, up to 200-300, or even more in number, lying in the cytoplasm, and presenting a more or less longitudinal polarity (figure 1); sometimes they lie free within the cytoplasm, but generally each of them lies in close contact with a large, weakly staining grain, apparently of the nature of a food storage product (figure 15), and usually they lie around the granule in the form of an incomplete ring.[1] Quite frequently one may observe two such mitochondria lying around one granule; at other times one sees only a single Y-shaped rod, and the fact that all intermediate stages from a single rod, though a Y- or V-shaped condition, up to two rods may be observed, seems to be good proof that the mitochondria are undergoing longitudinal fission on the surface of the vegetative granules. (figures 14 and 15).

During the binary fission of the binucleate organism the mitochondria appear merely to distribute themselves between the two resulting organisms. I have not been able to follow the process in fixed material, since fission is rarely seen. In figure 2, however, is shown an Opalina drawn from a living organism, which was undergoing longitudinal fission while in Ringer's fluid. The mitochondria under these conditions tend to concentrate, by migration, at the anterior and posterior ends, only a limited number being present in the mid-region of the animal. Even in fixed material specimens can often be obtained in which a definite indication of a migration process is to be observed (figure 1). Fission of the organism does not appear to be accompanied by a simultaneous division of the mitochondria, but the number of mitochondria is roughly halved by their distribution among the two resulting individuals. In the dividing organism shown in figure 2, the mitochondria, which are to be seen as somewhat strongly refractive bodies through its cuticle, have largely lost their rod-shape appearance. I am unable to state whether this occurs normally during binary fission, or whether it is a deleterious effect due to the Ringer's fluid which bathes them.

The resulting uninucleate organisms may then either develop into binucleate forms again, the mitochondria undergoing multiplication in the manner above described, or if conditions are suitable they will proceed to encystment, their size being considerably reduced, apparently, by further fission. In order to examine the process of encystment I have adopted the device of placing the organisms

[1] Metcalf, in his monograph on the Opalinids (3), does not specially mention mitochondria, and the methods used in his investigations (fixation with alcohol, corrosive sublimate, or formalin) would not permit of their detection; nevertheless, in many of his figures, cytoplasmic inclusions, having a close resemblance to the mitochondria in their spherical condition, are depicted. It is of particular interest to note that Metcalf observed an extrusion of granules to the exterior through the excretory pore, though their nature was not established; further, in referring to the Opalinids that arise from the spherical cysts, he states that they appear "in the same condition as that in which they enter the cyst, except that first they have usually extruded most of their ectosarc spherules."

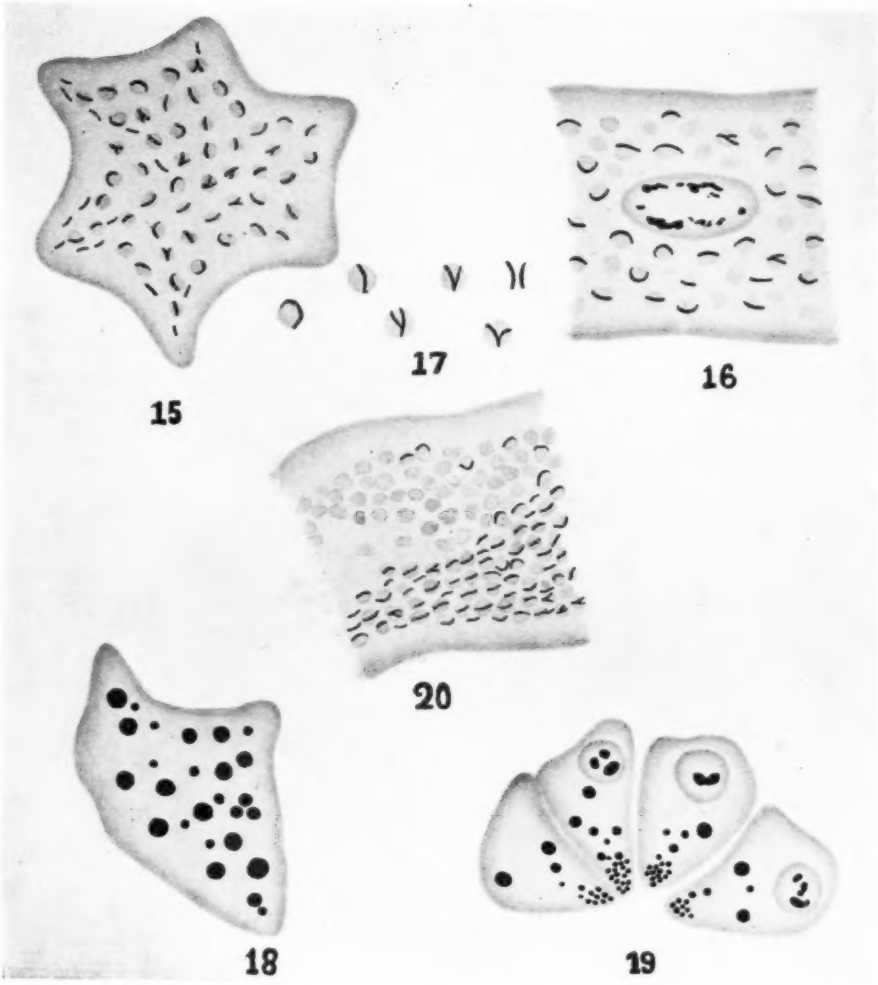
in Ringer's fluid. In this medium they become less and less active, and finally they round themselves off into cysts, which do not differ in appearance from the natural cysts that I have frequently obtained from the rectum of newly infected tadpoles, except that they are much larger than the latter, since they are formed by the rounding off of large binucleate forms. If such Ringer's cysts are sectioned and then stained, rod-like bodies are no longer present, but their place is taken by somewhat larger, deeply staining, spherical bodies. The larger vegetative granules have also disappeared, having apparently been absorbed into the general protoplasm of the organism.

Now if large numbers of Opalinas are induced to encyst by this method of placing them in Ringer's fluid, all stages between commencing and complete encystment are obtained. The process of rounding off of the organism is shown in figures 5-7, and need not be specially described here. When the mitochondria are examined in these developing cysts, we find that they have lost, or are rapidly losing, their rod shape, and most of them are already in the spherical condition. Before the encystment stage is arrived at one can also observe cases in which the mitochondria have swollen, and almost every one is undergoing what appears to be a transverse binary fission. This is shown quite clearly in figure 4, where most of the bodies are dumb-bell shaped, while others have already divided into two spheres. Mottier (4) describes a similar phenomenon in the cells of higher plants. In the protozoa, Fauré-Fremiet has described a similar process (1).

When we examine these Ringer cysts, and compare them with natural cysts from normally infected tadpoles, the former show considerably more of these rounded bodies than the latter; however, on account of the extraordinary difficulty of obtaining the Opalinas while these are undergoing natural encystment, I am unable to state whether such cyst formation is always preceded by fission of the mitochondria.

During the sexual phase of the life-cycle, when conjugation of the gametes occurs, a marked fusion of the rounded mitochondria seems to take place, since the rounded bodies as such are absent, and their place is taken by large clumps of granular material. The growth of the zygote is accompanied by a reformation of the mitochondria from these clumps of deeply staining material, but I am not able to give any details of the actual process by which this takes place. The mitochondria grow in size, large vegetative granules develop in connection with them, and their form, as seen in the fully developed adult binucleate organism, is attained.

Special significance attaches here to the occurrence of the vegetative granules and their connection with the mitochondria. In the uninucleate forms which arise from the zygote the mitochondria are already in the bent or ring-shaped rod condition; small granules are to be seen in close connection with them. As the organism grows, these granules grow also, and the mitochondria show a tendency to break away from them (figure 19). Now absorption of food



DESCRIPTION OF PLATE 2.

Sections of Opalina, fixed in osmo-chromic, stained with Heidenhain's iron-haematoxylin.

Fig. 15. Shows mitochondria each in close relation to large, rounded, vegetative granules. Several mitochondria undergoing fission. From transverse section of Opalina.

Fig. 16. The same, from longitudinal section.

Fig. 17. Mitochondria undergoing fission at surface of vegetative granule. On completion of fission they separate from granule.

Fig 18. Transverse section of Opalina after twelve hours' immersion in Ringer's fluid. The large globules have arisen by fusion of mitochondria.

Fig. 19. Corresponding change from pancreas of guinea-pig.

Fig. 20. Longitudinal section of Opalina. Many of the mitochondria have separated from the vegetative granules.

in Opalina consists solely in a diffusion of digested or digestible material through the cuticle into the cytoplasm; no solid material is taken into the body. From this it would appear that in Opalina a metabolism, involving the visible condensation of vegetative material, is occurring at the surface of the mitochondria. This observation seems to lend strong support to the speculation (2) that the mitochondria are loci of protein, and possibly of general protoplasmic synthesis, within the body of the living cell.

SUMMARY AND CONCLUSIONS.

1. Mitochondria are present in large numbers in Opalina at all stages of the life cycle.

2. In the asexual multiplicative phase of the life cycle they are bent, filamentous bodies, and multiply by longitudinal fission. Immediately preceding encystment they may divide transversely, and thus may produce spherical bodies in which they persist in the cyst and the gametes. In the zygote they appear to fuse into larger masses, which later break up into small granules again, which in their turn elongate to form, again, the filaments of the asexual form.

3. These observations afford strong evidence for the view that the mitochondria are persistent, self-reproducing bodies, and not metabolic products of the cytoplasm, though the latter possibility has not been disproved.

4. Evidence is given that synthesis of vegetative granules (storage products) may take place at the surface of the mitochondria.

I am greatly indebted to Dr. O. W. Tiegs, of Melbourne University, for his help and suggestions.

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EXPERIMENTAL STUDIES ON GROWTH.

XVIII. FURTHER EXPERIMENTS ON THE INFLUENCE OF TETHELIN UPON THE GROWTH AND LONGEVITY OF THE WHITE MOUSE.[1]

By

T. BRAILSFORD ROBERTSON and L. A. RAY.

(From the Darling Laboratories of Physiology and Biochemistry, University of Adelaide).

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Results Previously Obtained.

We have previously shown (5, 10, 11) that tethelin (the ether-precipitable fraction of an alcoholic extract of the anterior lobe of the pituitary body), when administered to mice by mouth in dosage of 4 milligrammes daily throughout the duration of life, causes initial retardation of growth, followed by acceleration, which, however, never fully compensates the initial retardation. The animals remain permanently lighter in weight than the control normals, but as their growth in linear dimensions is more than proportionally retarded, they are exceptionally heavy animals for their size. Animals of like size, therefore, are heavier than normal animals, while animals of like weight are smaller than normal. The mean duration of life is very much prolonged, the duration of life of the treated animals being 99 days, or 13 per cent. in excess of that of the normals.

In another experiment which was carried out at the same time, the administration of tethelin was discontinued from time to time because sufficient tethelin was not then procurable (in 1914) to supply the requirements of both experiments. The administration of this substance was, for this reason, confined to three periods of four weeks each during the first thirty weeks of life, the dosage being, as in the accompanying experiment, 4 milligrammes per diem, and the channel of administration the mouth. Notwithstanding the great difference between the total amounts of tethelin administered to the two groups of animals, and the comparatively small proportion of their lives during which it was administered to the second group, the effect upon the growth of the animals fell very little short of the effect of continuous administration. Growth was initially

[1] The expenses of publication of this article were in part defrayed by The Animal Products Research Foundation.

retarded, and the subsequent acceleration failed by a considerable margin to re-establish normal weight. The duration of life was 81 days, or 11 per cent. in excess of normal. Of six other groups of 36 animals, each selected at random from the same stock, only one exhibited a duration of life in excess of normal, and that group consisted of male animals in receipt of fresh ox-pituitary (one-twelfth of an anterior lobe daily) corresponding to about 1 mg. of tethelin. The excess over normal life-duration in this group, however, was only 25 days, and may easily have been fortuitous, more especially since females similarly treated did not display any enhancement of viability whatever.

The animals selected for the second experiment were not taken from the same litter as those selected for the first experiment. The two groups represented, therefore, two unrelated random samplings from the same stock. From the probable errors of the observed life-durations of the normal and treated animals the separate chances that each observed excess of viability was accidental may be computed. In the first experiment the chance was 1 in 22·25, in the second 1 in 6·75. The chance that *both* were accidental is in the customary manner of computing joint probabilities, the product of the two separate chances, namely, one chance in one hundred and fifty.[2]

The possibility, thus indicated, of eliciting important and enduring effects of tethelin upon the growth and viability of animals by means of comparatively brief periods of administration, suggested inquiry into the effect of a single brief period of administration in early life. Accordingly, in 1916 tethelin was administered to mice by mouth in dosage of 4 milligrammes per day for a single brief period, from the fourth to the twelfth weeks of age inclusive. The results are reported in the fourteenth and fifteenth papers of this series (12, 13). Growth was initially retarded, as in the previous experiments, but the secondary acceleration of growth was far more pronounced than it was in the animals which received the tethelin continuously, so pronounced, indeed, that it led to a considerable excess of accretion of weight, which attained a maximum at about 500 days of age, when the treated animals exceeded the normals by no less than 25 per cent. of their weight. By this means, therefore, a condition had been brought about in animals which appeared analogous to human gigantism of pituitary origin. A similar degree of gigantism was subsequently induced by Uhlenhuth in *Amblystoma* larvae by the administration of fresh pituitary tissue (15).

The viability of the animals which received tethelin for the single brief period was not increased; it was, in fact, slightly inferior to that of the control

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[2] Pearl has questioned the justifiability of this computation (4). His objection, however, appears to be based upon the mistaken belief that the second group of animals was composed of females derived from the same litters as the males employed in the first experiment. Had this been the case, then the two groups could not have been properly considered to be unrelated random samples, and this fact would have vitiated the theoretical basis of the computation.

animals. The experiment was repeated in 1917, with similar initial retardation and exaggerated compensatory acceleration of growth, but the experiment was terminated before the decease of all of the animals, on account of our removal from Toronto to Australia. Another experiment on the continuous administration of tethelin by mouth again showed that the continued administration of this substance diminishes and delays the compensatory acceleration of growth which succeeds the initial retardation.

It appeared from these experiments, therefore, that the main effect of tethelin is to induce retardation of growth. The secondary acceleration of growth seems to be attributable, not directly to the tethelin, but to compensatory factors which develop in the animal itself in response to the abnormal dosage of the active principle of the anterior lobe of the pituitary body. Tethelin is capable of retarding growth due to these factors no less than normal growth, so that if its administration is continued the compensatory overgrowth is slight. If, however, the administration of tethelin is discontinued before these compensatory growth-factors come into play, then the growth which they induce remains unchecked, and results in eventual gigantism.

On the other hand, tethelin does not retard the growth of every kind of tissue or cell. On the contrary, the growth of epithelial tissues is accelerated. Thus the growth of inoculated Flexner-Jobling carcinoma is accelerated by administration of tethelin to the rats (8), while the direct application of tethelin to superficial wounds hastens repair through local acceleration of the growth of epithelium (1, 2, 3, 6). Tethelin also enhances the division rate of infusoria (7). The retardation of the growth of animals, as a whole, therefore, is probably attributable to retardation of the growth of certain relatively bulky tissues other than epithelium, either directly, or indirectly, through the promotion of the growth of tissues which contribute less to the weight and bulk of the animal.

New Experiments.

In the experiments on the influence of tethelin upon the growth of mice which are enumerated above, the substance was administered with the food by mouth. This method of administration might very conceivably lead to loss, either through destruction by digestion in the alimentary canal, or by failure to absorb the whole of the material ingested. It thus appeared possible that subcutaneous administration of the same dosage might result in enhancement of its effects upon the growth of the animals. Accordingly, in 1922, twenty-four females of five weeks of age were selected at random from a number of litters born within a week of one another, and to these 4 milligrammes of tethelin was administered daily (excepting Sundays). The tethelin employed was that manufactured by H. K. Mulford Co., of Philadelphia, and was packed in evacuated tubes, each containing 100 mg. of substance. One tube, therefore,

sufficed for each day's treatment. The contents of the tube were dissolved in 6 cc. of sterile Ringer's solution and 0·25 cc. of this solution was administered to each mouse, in the right and left axillae alternately, the skin being previously washed with alcohol.

Thirty-six females were also selected at random to serve as controls from litters of the same generation, but not from the same litters as those from which the experimental animals were obtained. The reason for this was that in order to economize tethelin (since it is subject to decomposition after the tube is opened), it was necessary to begin and cease treatment of all of the experimental animals simultaneously. Otherwise, if one-half of the animals had been a week older than the other half, for example, it would have been necessary to begin treatment of the older animals a week before the others, and to continue treatment of the younger animals for a week longer than the older animals. For two weeks, in that case, half a tube of tethelin would have been wasted daily. It was not found possible to obtain more than twenty-four females from our breeding-stock in one week. To have obtained the thirty-six controls as well from the same litters would have necessitated a breeding-stock far larger than that which we have found practicable to maintain. On the other hand, it has been shown in the preceding article of this series (14) that the custom of choosing experimental and control animals alternately from the same litters is devoid of either experimental or theoretical foundation, since animals of a given generation differ no more among one another than the animals of a single litter.

The treatment was continued for eight weeks, from the fifth to the thirteenth weeks of age inclusive. During the first three weeks of treatment it was noted that small lesions of 1 or 2 mm. diameter were frequently formed at the site of injection, due possibly to laceration by the needle or to the compression applied with forceps to prevent leakage after withdrawal of the needle. The animals, as stated, were injected on the right and left sides alternately, so that forty-eight hours after injection we again sought to inject on the same side. By this time the lesions were almost invariably found to have healed. At the same time we were injecting a thymus extract into twenty-four other animals once a week,[3] and we intended injecting always on the same side. Quite frequently, however, we were compelled to change the side of the injection because the lesions inflicted a week ago were not yet healed. After the fourth week the animals receiving tethelin no longer exhibited any visible lesions at all. Our observations thus afford confirmatory evidence of the effect of tethelin in accelerating the repair of superficial lesions.

The animals, both experimental and control, were weighed once a week for the first thirty weeks, and once a fortnight thereafter for the entire duration

[3] This extract contained only a trace of protein. The results of this experiment have not yet been reported.

of their lives. The technique of the experiments and the diet employed were those which have been fully described in previous publications of this series (9, 14).

Two of the animals died within a few days of their introduction into the experimental cages, the cause of death being the (probably non-infective) enteritis, to which reference is made in the preceding paper of this series (14). In any other experiment these would have been replaced by fresh animals; but since it was necessary, in order to economize the tethelin, as explained above, that all of the animals should be as nearly as possible of the same age, in this experiment the animals thus lost could not be replaced. Control animals similarly lost were replaced by others as usual.

At postmortem examination, but not until after the lapse of over a year, it was found that four animals in one compartment had been suffering from a polycystic infection of the liver with *Cysticercus fasciolaris*.[4] The infection of these animals unquestionably dates from the brief introduction of a kitten into the laboratory. The kitten was doubtless placed upon the top of the cage by some one of the laboratory assistants, with the result that the animals in this compartment were infected. No other animals in this experiment were infected, nor were any of the controls, and apart from one or two sporadic cases of individuals having single cysts, doubtless originating from the same source, no other cases have been encountered among our animals in this laboratory.[5] Since it was necessarily uncertain to what extent the welfare of these animals had been deleteriously affected by the very heavy infection which they had sustained, and, indeed, it was impossible to estimate what proportion of their weight was actually attributable to the cysts, the weighings of these animals were deleted from the experimental records. With these losses and deletions, therefore, the total number of experimental animals was reduced to eighteen.

The results of the experiment are enumerated in Tables 1 to 4 inclusive, and are graphically represented in figure 1. The principles upon which the figure is constructed are fully explained in the preceding article of this series (14), but in brief the growth of each group of animals is represented not, as usual, by a curve passing through the ascertained average weights, but by a shaded area, of which the centre at any age is the ascertained average weight and the width is twice the "probable error" of the average. If two such areas intersect they are to be regarded as identical. The period of administration of the tethelin is indicated by the length of the line in the lower left-hand corner of the diagram.

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[4] We are indebted to Professor Harvey Johnston for the identification of this worm.

[5] Cysticercus infection was occasionally found, as previously reported, among our stock in California (9).

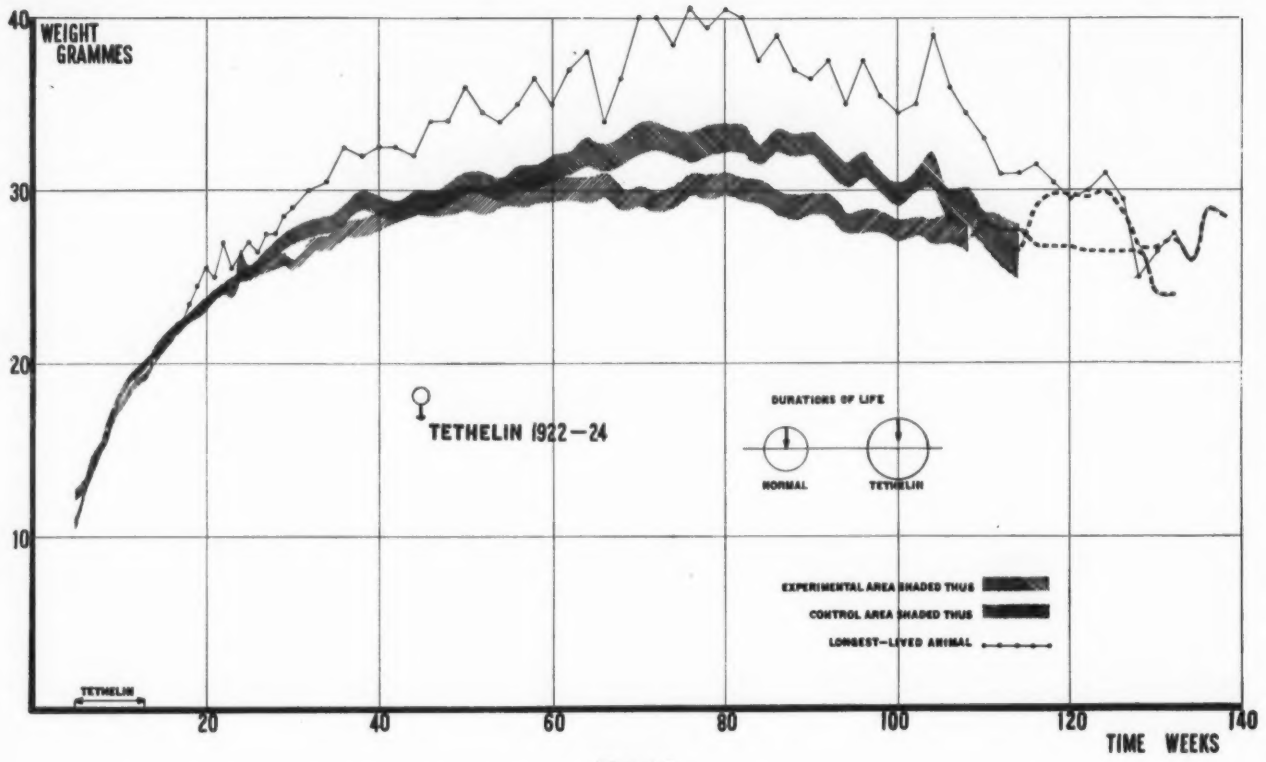

Figure 1.

Comparing the growth of Normal Female Mice with that of Female Mice of the same generation which received pre-adolescent treatment with 4 milligrammes of tethelin per diem from the fifth to the thirteenth weeks of age inclusive, administered hypodermically. The centres of the shaded areas represent the average weights of the two groups, their widths twice the probable errors of these averages. The centres of the circles are situated at the average durations of life of the two groups, and their diameters are twice the probable errors of these averages.

It will be seen that the growth of the treated animals is appreciably retarded in comparison with the controls for the first ten weeks. At about twenty-five weeks the compensatory acceleration carries their average weight above that of the controls, until the fortieth week. From the fortieth to the sixtieth week the curves are identical (within the experimental error), but after the sixtieth week and until the hundred and fifth week the compensatory overgrowth of the treated animals renders them definitely superior in weight to the controls, the maximum deviation of the two groups occurring at about 70 weeks, when the treated animals exceeded the controls by 3·33 grammes, or 11·2% of the normal average weight at that age.

The average durations of life are represented by the centres of the circles placed upon a line parallel with the time-axis. The radius of each circle is the probable error of the average. It will be observed that the life-duration of the treated animals exceeded that of the controls by 90 days, a period far in excess of the combined probable errors of the measurements, and, curiously enough, almost exactly the prolongation of life-duration which was obtained in previous experiments, outlined above, in which the tethelin was administered by mouth continuously or intermittently for a considerably longer period than the period of administration employed in these experiments.

In order to ascertain whether the deformation of the growth-curve and the prolongation of the life-duration are essentially and not merely adventitiously associated, the growth-curve of the longest-lived animal which was treated with tethelin was separately plotted in the diagram. The duration of life of this animal (Eb3) was 1,036 days (148 weeks), and it will be seen that it displayed to an exaggerated degree the departures from the normal growth curve which characterize the average of the treated animals. The longest-lived animal among the controls only attained the age of 934 days. In our experience no normal female of our Australian stock has ever attained 1,000 days' duration of life. The second longest-lived animal of the treated group (Eh2) attained the age of 925 days, which is not outside the possible range of life-duration of normal females. Its curve of growth coincided with that of the longest-lived animal for the first thirty weeks, and thereafter approximated to the average curve for the treated group.

In the accompanying plate (figure 2) are shown photographs of all of the surviving treated animals (distinguished by the large letter E) at 500 days of age (71 weeks), when the deviation of the treated from the control animals was at a maximum. The survivors of only twenty-four of the controls are shown (distinguished by the large letter C), those, namely, which inhabited cages distinguished by the same letters (b, d, f, and h) as those inhabited by the experimental animals. Since this lettering is perfectly arbitrary, the animals of which the photographs are shown represent a random choice among all of the surviving controls. The animals are photographed upon a black background

divided into square centimeters by white lines. The background was photographed first, and the animal then placed upon it and photographed upon the same plate, so that the squares appear through the photograph of the animal. A Graflex camera, provided with a Cooke-Aviar lens of 7 inch focal length, was employed, and the exposure, in diffused daylight before an open window, varied between one-tenth and one-fifteenth of a second, so that movements of the animals rarely interfered with the success of the photographs. Two normals (Cb1 and Cf6) were, however, only one year old when the photographs shown were taken. The reason for this is that these animals died very shortly after the attainment of the age of 500 days (535 and 548 days respectively), and had already exhibited premortal loss (Cb1) or gain (Cf6) of weight when the photographs of the other animals were taken. The animal Cb1 lost 5·5 grammes just before death, and the animal Cf6 gained 2·5 grammes. Had photographs of these animals been taken at 500 days, therefore, they would not have afforded a true representation of their appearance during the period immediately preceding the sudden premortal changes of weight. During the interval from one year to nearly 500 days, however, these animals only slightly increased in weight, and their photographs at one year are therefore not far from a true picture of what their condition would probably have been at 500 days had disease not supervened.

Inspection of this plate at once shows that the linear dimensions of the treated animals, estimated by their projected areas, were in most cases considerably superior to the dimensions of the controls. Our estimates of the projected areas of each of the animals represented (exclusive of projecting appendages, namely, ears, feet, and the hairless part of the tail) are enumerated in Table 5. The average projected area of the fifteen surviving animals of the treated group is 28·1 square centimeters, with a standard deviation of 2·5 square centimeters and a variability of 8·8%. The average projected area of the nineteen surviving animals of the control group which are represented in the plate is 24·9 square centimeters, with a standard deviation of 2·1 square centimeters and a variability of 8·4%. The average projected area of the treated animals exceeds that of the controls, therefore by 12·85%, or slightly more than their average weight exceeds that of the controls. The weight of the treated animals per square centimeter of projected surface is 1·178 grammes, while that of the control animals is 1·184 grammes. The bodily density or "ponderal index" of the two groups of animals is therefore, within the error of estimation, the same.[6]

[6] The attitude of the animals at the moment of photographing does not appreciably effect the accuracy of this estimate. Retracted or "hunched up" animals compensate for the diminution of projected length by the increase of projected width, and several estimations of the projected areas of the same animals in different positions have yielded results which are identical within the attainable accuracy of the estimates. Often, in taking a series of photographs of animals it has been found that the photograph of one animal has been duplicated, or, again, photographs are often taken twice of the same animal, in case some movement (generally in response to the click of the shutter, and therefore occurring *after* exposure) may have spoiled the first picture. These duplicate photographs were employed for testing the influence of attitude upon the accuracy of the estimate of projected area.

Inspection of the plate also reveals the fact that the experimental animals were very unequally affected by the treatment, notwithstanding the fact that this was identical for all. Table 5, however, shows that of the treated animals only one out of fifteen (Eh4) fell below the *average* normal in projected area, while of the control animals only three out of nineteen attained the *average* projected area of the treated animals. The two groups, therefore, are sharply distinguishable, although the largest controls overlap to a certain extent the lower portion of the range of variability in size of the treated animals.

Discussion of Results.

The effect of the hypodermic administration of tethelin in dosage of 4 milligrammes per day for eight weeks, from the fifth to the thirteenth weeks of age inclusive, upon the growth of female white mice is intermediate between the effects previously obtained by administration of the same dosage by mouth for brief (eight weeks) and more prolonged periods respectively. The initial retardation of growth is less than that previously observed, although definitely appreciable. The subsequent compensatory acceleration of growth is greater than that obtained in response to prolonged administration by mouth, but less than that obtained in response to brief administration by mouth. Whether this difference is due to the difference in the route of administration or to genetic differences in the stocks of animals employed, or to differences in the preparation of tethelin administered, cannot at present be decided. The maximum deviation of weight from the controls occurred at precisely the same age (500 days) as in the previous experiment, in which administration by mouth from the fourth to the twelfth weeks was employed. The average duration of life of the treated animals was prolonged to almost precisely the same extent (90 days) as in the previous experiment, in which administration by tethelin by mouth was continued throughout the duration of the lives of the animals. The treated animals exceeded the controls in size, but the bodily density of the two groups was identical, whereas continuous administration of tethelin by mouth produces animals which are inferior to normals in size but exceed them in bodily density. Thus, in its effects upon growth and bodily density, the results of hypodermic administration of tethelin for eight weeks (from the fifth to the thirteenth weeks inclusive) resemble the results of administration by mouth in like dosage and for a like period, but in its effects upon the average duration of life the result resembles that of more prolonged administration by mouth.

CONCLUSION.

Preadolescent hypodermic administration of tethelin to mice, in dosage of 4 milligrammes per day, causes initial retardation, followed by compensatory

acceleration of growth, which eventually leads to a considerable overgrowth of the treated animals, which attains its maximum at about 500 days of age. The treated animals then exceed the normals both in size and in weight. The average life-duration of the treated animals exceeded that of the controls by 90 days.

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TABLE 1.

GROWTH OF NORMAL FEMALE WHITE MICE, 1922-1924.

No. of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
36	5	12·39	± 0·33
36	6	12·93	± 0·32
36	7	13·89	± 0·30
36	8	15·18	± 0·31
36	9	16·71	± 0·36

No. of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
36	10	18·07	± 0·30
36	11	18·97	± 0·25
36	12	19·43	± 0·24
35	13	19·93	± 0·27
35	14	20·69	± 0·27
35	15	21·20	± 0·27
35	16	21·86	± 0·27
35	17	22·21	± 0·27
35	18	22·60	± 0·30
35	19	23·10	± 0·32
35	20	23·51	± 0·32
35	21	23·94	± 0·33
35	22	24·33	± 0·34
35	23	24·59	± 0·33
35	24	24·99	± 0·37
35	25	25·10	± 0·35
35	26	25·39	± 0·34
35	27	25·64	± 0·39
35	28	25·80	± 0·40
35	29	26·01	± 0·37
34	30	25·63	± 0·31
35	32	26·73	± 0·42
35	34	26·90	± 0·46
35	36	27·70	± 0·48
35	38	27·79	± 0·52
33	40	28·21	± 0·58
33	42	28·67	± 0·62
32	44	29·31	± 0·64
32	46	29·16	± 0·68
32	48	29·27	± 0·72
32	50	29·58	± 0·78
32	52	29·33	± 0·77
32	54	29·83	± 0·80
32	56	29·80	± 0·79
32	58	30·06	± 0·77
32	60	30·08	± 0·72
32	62	30·05	± 0·72
31	64	29·98	± 0·74
30	66	30·33	± 0·72
28	68	29·39	± 0·64
27	70	29·67	± 0·58

No. of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
25	72	29·32	± 0·59
24	74	29·63	± 0·65
22	76	30·32	± 0·63
22	78	30·20	± 0·66
22	80	30·39	± 0·63
20	82	30·03	± 0·69
20	84	29·93	± 0·69
19	86	29·29	± 0·69
19	88	29·00	± 0·72
16	90	29·22	± 0·61
14	92	29·14	± 0·66
12	94	28·04	± 0·56
11	96	28·23	± 0·67
10	98	28·20	± 0·63
10	100	27·65	± 0·63
8	102	27·94	± 0·54
6	104	27·58	± 0·75
6	106	27·83	± 0·87
6	108	27·17	± 0·72
4	110	28·13	—
4	112	27·78	—
4	114	27·78	—
3	116	26·83	—
2	118	26·75	—
2	120	26·75	—
1	122	26·50	—
1	124	26·50	—
1	126	26·50	—
1	128	26·50	—
1	130	24·00	—
1	132	24·00	—

TABLE 2

GROWTH OF FEMALE WHITE MICE WHICH RECEIVED 4 MILLIGRAMMES OF TETHELIN DAILY FROM THE FIFTH TO THE TWELFTH WEEKS OF AGE INCLUSIVE, 1922-1924.

No. of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
18	5	10·72	± 0·29
18	6	12·28	± 0·29

No. of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
18	7	14·39	± 0·23
18	8	15·08	± 0·30
18	9	16·36	± 0·22
18	10	17·28	± 0·23
18	11	18·17	± 0·25
18	12	18·81	± 0·26
18	13	19·17	± 0·26
18	14	20·11	± 0·26
18	15	20·75	± 0·26
18	16	21·42	± 0·24
18	17	21·97	± 0·26
18	18	22·61	± 0·24
18	19	22·83	± 0·26
18	20	23·33	± 0·27
18	21	23·92	± 0·27
18	22	24·28	± 0·29
11	23	24·05	± 0·29
7	24	25·79	± 0·78
18	25	25·28	± 0·40
18	26	25·61	± 0·42
18	27	26·25	± 0·38
18	28	26·58	± 0·46
18	29	27·00	± 0·48
18	30	27·31	± 0·48
18	32	27·75	± 0·52
18	34	28·00	± 0·50
18	36	28·67	± 0·53
18	38	29·42	± 0·52
18	40	29·11	± 0·57
18	42	28·81	± 0·56
18	44	29·25	± 0·67
18	46	29·36	± 0·61
18	48	29·78	± 0·70
18	50	30·44	± 0·65
18	52	30·47	± 0·67
18	54	30·06	± 0·66
18	56	30·83	± 0·64
18	58	30·81	± 0·72
17	60	31·32	± 0·74
17	62	31·62	± 0·66

No. of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
16	64	32·28	± 0·82
16	66	31·94	± 0·80
15	68	32·37	± 0·86
15	70	33·00	± 0·92
15	72	33·17	± 0·86
15	74	32·83	± 0·77
15	76	32·57	± 0·90
14	78	32·93	± 0·90
14	80	33·04	± 0·87
14	82	33·07	± 0·82
14	84	32·07	± 0·58
13	86	32·92	± 0·67
13	88	32·65	± 0·66
12	90	32·79	± 0·64
11	92	31·82	± 0·70
11	94	30·91	± 0·74
11	96	31·50	± 0·71
11	98	30·50	± 0·62
9	100	29·78	± 0·71
8	102	30·50	± 0·65
7	104	31·21	± 1·04
7	106	29·07	± 1·01
6	108	29·08	± 1·13
6	110	27·83	± 1·00
5	112	27·10	± 1·50
5	114	26·50	± 1·58
4	116	29·13	—
3	118	29·83	—
3	120	29·83	—
3	122	29·67	—
2	124	30·00	—
2	126	28·75	—
2	128	26·75	—
2	130	26·75	—
2	132	27·25	—
1	134	26·00	—
1	136	29·00	—
1	138	28·50	—
1	140	28·50	—
1	142	27·00	—
1	144	26·50	—
1	146	21·50	—

TABLE 3.

MORTALITY STATISTICS FOR NORMAL FEMALE WHITE MICE, 1922-24.

(Accidental deaths excluded.)

Age Period.	Per cent. of all Animals dying within this period.	Per cent. of Survivors dying within this period.	Per cent. of all Animals dead at end of this period.
210-500 days	25·7	25·7	25·7
500-600 „	20·0	26·9	45·7
600-700 „	25·7	47·4	71·4
700-800 „	17·1	60·0	88·5
800-900 „	8·6	75·0	97·1
900-1000 „	2·9	100·0	100·0

Mean duration of life: 610 ± 18 days.

TABLE 4.

MORTALITY STATISTICS FOR FEMALE WHITE MICE WHICH RECEIVED 4 MILLIGRAMMES OF TETHELIN DAILY FROM THE FIFTH TO THE TWELFTH WEEKS OF AGE INCLUSIVE, 1922-24.

(Accidental deaths excluded.)

Age Period.	Per cent. of all Animals dying within this period.	Per cent. of Survivors dying within this period.	Per cent. of all Animals dead at end of this period.
210-500 days	16·7	16·7	16·7
500-600 „	11·1	13·3	27·8
600-700 „	11·1	15·4	38·9
700-800 „	38·8	63·6	77·7
800-900 „	11·1	50·0	88·8
900-1000 „	5·6	50·0	94·4
1000-1100 „	5·6	100·0	100·0

Mean duration of life: 700 ± 25 days.

TABLE 5.

COMPARING THE PROJECTED AREAS OF TREATED AND CONTROL ANIMALS AT 500 DAYS OF AGE.

Control Animals.		Treated Animals.	
Animal Number.	Projected Area in sq. cm.	Animal Number.	Projected Area in sq. cm.
Cb1	23·35	Eb1	27·90
Cb2	26·40	Eb2	27·50
Cb3	24·65	Eb3	31·90
Cb4	23·70	Eb5	28·45
Cb5	22·20	Ed5	26·80
Cb6	28·55	Ef1	28·30
Cd2	23·70	Ef2	27·25
Cd3	26·70	Ef4	34·80
Cd4	24·60	Ef6	28·35
Cd5	23·00	Eh1	27·05
Cd6	28·25	Eh2	25·20
Cf2	23·70	Eh3	27·05
Cf3	28·25	Eh4	23·90
Cf4	25·80	Eh5	29·55
Cf5	20·35	Eh6	27·20
Cf6	24·00		
Ch1	24·40		
Ch4	25·95		
Ch6	25·65		

Average Area of Controls = 24·91 sq. cm.

Average Area of Treated Animals = 28·08 sq. cm.